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Control of Traumatic Liver Hemorrhage in the Cirrhotic Rat by Intraportal Infusion of Norepinephrine*

C. Vagianos, S. Steen, E. Zoucas, H. Asakawa, B. Jeppsson, and S. Bengmark

Dept. of Surgery, Lund University, S-221 85 Lund, Sweden

Summary. The effect of intraportal infusion of norepinephrine (NE) on primary hemostasis in the cirrhotic rat was investigated at standardized liver trauma. Cirrhosis was induced by simultaneous administration of increasing amounts of carbontetrachloride (CCl_4) and phenobarbitone. Infusion of norepinephrine took place after cannulation of the gastroduodenal vein. Intraportal infusion of NE resulted in a significant increase in arterial blood pressure and portal pressure in all animals. No difference was observed between cirrhotic and control rats.

Cirrhotic animals bled longer and more profusely as compared with the controls. Infusion of NE resulted in significant decrease in bleeding time and blood loss. NE did not affect hematocrit, hemoglobin, platelet, or white cell count. Platelet aggregation was not influenced by the compound. In conclusion, intraportal infusion of NE proved effective in decreasing hemorrhage at liver trauma in cirrhotic rats.

Key words: Cirrhosis – Liver trauma – Hemorrhage – Norepinephrine

Introduction

Cirrhosis has been associated with bleeding diathesis due to decreased production of coagulation factors. Simultaneously, depletion of essential fatty acids was shown to precipitate impairment of prostaglandin production and defective platelet function [1].

Liver surgery in the presence of cirrhosis has often been complicated by a high intraoperative and postoperative risk of hemorrhage. In cirrhotics, even average size operation carried an increased mortality of 10% due to bleeding [2, 3]. Earlier studies have shown that intra-arterial or intraportal infusion of norepinephrine successfully controlled bleeding after standardized liver trauma in the rat [4]. Using the same experimental model, the present experiments

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Offprint requests to: Evita Zoucas, MD, PhD (address see above)

aimed at investigating the effect of norepinephrine at hepatic trauma in cirrhotic animals.

Materials and Methods

Male Sprague-Dawley rats with an initial body weight of 100–150 g were used and maintained on a stock pellet diet.

Cirrhosis was induced according to Proctor and Chatamra [5, 6, 7] by simultaneous administration of CCl_4 and phenobarbitone. Crystalline phenobarbitone was diluted in tap water to a concentration of 350 mg/l. This was the only drinking water available to the animals throughout the whole experiment. After 14 days of phenobarbitone, when the rats weighed about 220 g, CCl_4 administration began. CCl_4 was administered once a week using an intragastric tube (feeding tube, Argyle Ch-5, 38 cm, Sherwood, Republic of Ireland), under light ether anesthesia. Under the same conditions controls received the same amount of normal saline once a week. The initial dose of CCl_4 was 0.04 ml, and the subsequent doses increased up to a maximum of 0.4 ml according to the weight change. Body weight was carefully measured twice a week and was used as the main criterion for the treatment. The whole treatment with CCl_4 lasted 10–11 weeks.

The controls received phenobarbitone in drinking water for the same time as the CCl_4-treated animals.

Main proof of cirrhosis development were positive histological tests of liver. Portal pressure, presence of ascites, liver and spleen weight were also recorded in all the animals.

All animals were paired at random and ascribed to six groups according to the experimental procedure applied:

Group 1: Cirrhotic animals (16)
Group 2: Cirrhotick animals subjected to intraportal infusion of NE (21)
Group 3: Cirrhotic animals subjected to intraportal infusion of normal saline (13)
Group 4: Control animals (16)
Group 5: Control animals subjected to intraportal infusion of NE (21)
Group 6: Control animals subjected to intraportal infusion of normal saline (13).

Experimental Procedures

Ether anesthesia was used in all experiments. The femoral artery was cannulated in all animals using a polyethylene catheter (0.7 mm Portex, Hythe, Kent, UK), advanced to the aorta and connected to a transducer (EMT 34, Siemens-Elema, Sweden) for continuous registration of the arterial blood pressure (BP).

The abdomen was opened via a midline incision. In all animals the gastroduodenal vein was identified, dissected under an operative microscope (Zeiss OPMI 65) at ×25–40, and cannulated by a soft silicon catheter (602-105, Silastic, Dow Corning Co., MI, USA). Special care was taken so that the tip of the catheter did not project into the lumen of the portal vein, obstructing the portal flow. In Group 1 and 4 the intraportal catheter was used only for measuring the portal pressure. In Groups 2 and 5, after measuring the portal pressure, the catheter was used for continuous infusion of NE (Sigma Chemicals, St. Louis, MO, USA), 10^{-4} *M* at a rate of 10 ml/h using a Harvard pump. In Groups 3 and 6, 0.9% NaCl was infused at the same rate. Five minutes after infusion had started, a standardized liver trauma was performed. Trauma involved resection of the tip of the left anterior liver lobe amounting to 2.5%–3.5% of the total liver weight. The traumatized surface was observed through an operating microscope at ×10. Bleeding time, recorded as the time from trauma until hemostasis on the severed surface was completed, was evaluated. The extravasated blood, during the above period was collected in an aluminum cup, placed under the left liver lobe, and weighed [8].

Five animals from Groups 2, 3, 5, and 6 were used for the evaluation of portal pressure upon intraportal infusion of NE after insertion of a silicon catheter to the superior mesenteric vein.

Eight animals from Groups 1, 2, 4, and 5 were exanguinated by puncture of the aorta at times corresponding to the time of the liver trauma. Blood drawn upon exanguination was used for the determination of hemoglobin (Hb), hematocrit (Ht), platelet count (PC), white blood count (WBC), APT-time (APT-t), total serum protein, and platelet aggregation.

As no effect of saline infusion on the above mentioned parameters was expected, no separate groups of normal saline-infused animals were included in this part of the study.

Hb was determined according to van Kampen and Zijlstra [9], microhematocrit was determined in duplicate, PC and WBC were determined in a Bückner chamber, using a phase-contrast microscope. APT-time was estimated using the commercial standardized reagent (Cephotest, Nyegaard, Denmark). Platelet aggregation was assayed by the method of Born, using platelet-rich plasma (750×10^9 pl/l). Total serum protein was estimated by using the Biuret method [10].

The results were statistically evaluated by Wilcoxon's rank sum test and Student's *t*-test.

Results

A total of 93 rats were used for the induction of cirrhosis. The yield of histologically proved micronodular cirrhosis was 75% (70 animals), with a mortality of 12% (11 animals). Ascites developed in 47% of the cirrhotic rats (33 animals). Ten of the cirrhotic animals were used for the establishment of the model. Another ten were discarded because of technical errors.

Figure 1 shows the typical histological picture of the liver in a CCl_4-treated animal. Cirrhosis was prominent with several small regeneration nodules.

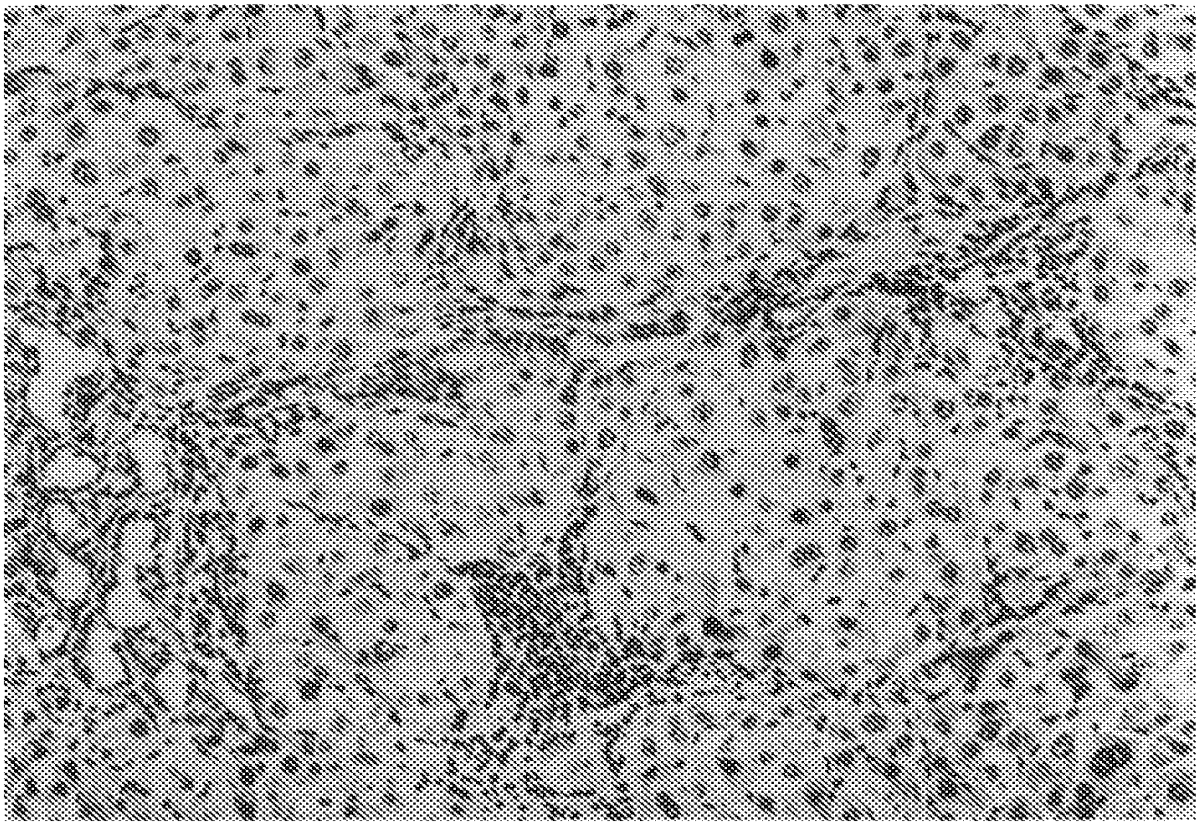

Fig. 1. Cirrhosis with several small regeneration nodules in CCl_4-treated animals. Proliferating bile ducts to the *left*. HE $\times 160$

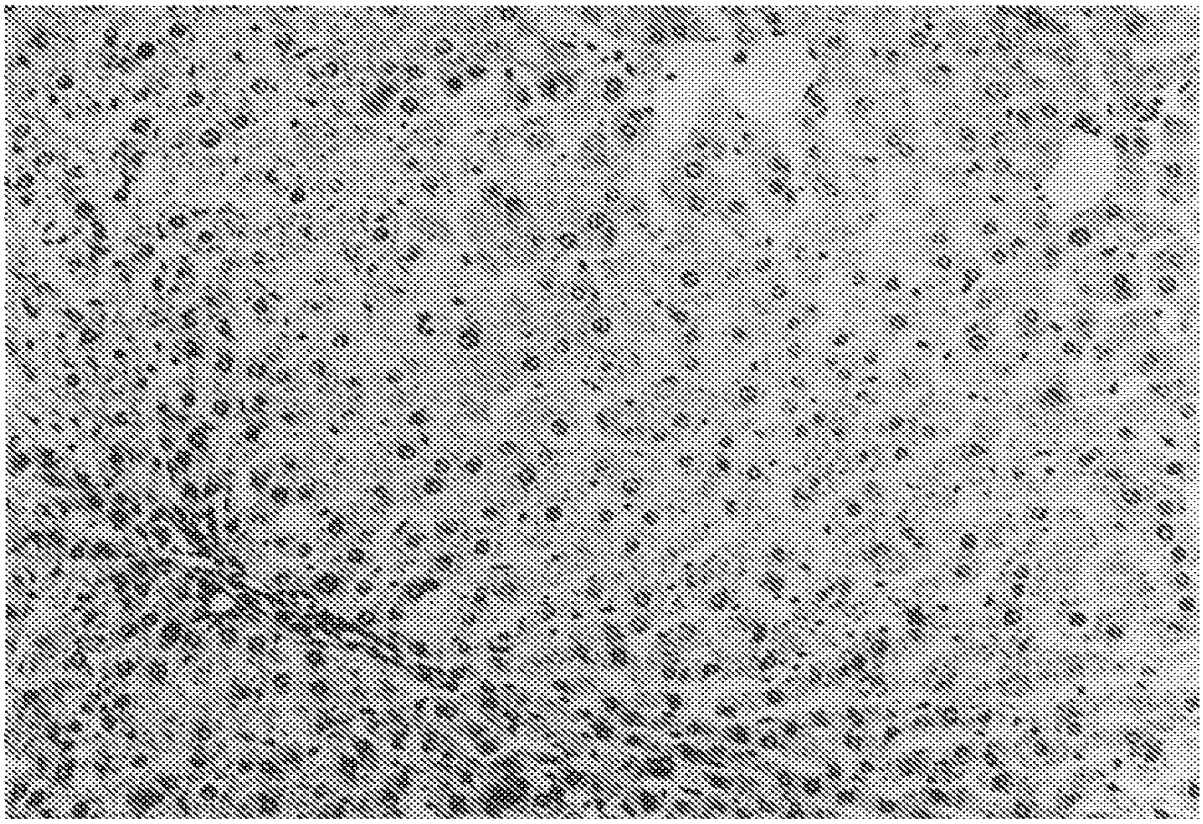

Fig. 2. Histological picture of control animal. Normal architecture with portal tract to the *lower left* and central vein to the *upper right.* HE × 160

Table 1. Body weight, liver weight, spleen weight, and portal pressure in control and cirrhotic animals

Group	No. of animals	Body weight (g)	Liver weight (% b.wt.)	Spleen weight (% b.wt.)	Portal pressure (mm Hg)
Cirrhotic	40	405.7 ± 8.5	4.5 ± 0.14	0.38 ± 0.02	12.8 ± 0.56
Controls	40	454.6 ± 5	4.7 ± 0	0.2 ± 0	6.9 ± 0.3
		NS	NS	s_1	s_1

Significance of difference, P $s \leq 0.01$, $s_1 \leq 0.05$
Values presented as mean ± SEM
Student's t-test

Figure 2 shows a liver specimen from a control animal. No cirrhotic changes were encountered.

As depicted in Table 1, body weight and liver weight did not differ in control and cirrhotic animals. Spleen weight amounted to 0.38% of the body weight in cirrhotic rats and only 0.2% b.wt. in controls. Portal pressure was significantly increased from 6.9 mm Hg in controls to 12.8 mm Hg in cirrhotics.

The initial blood pressure was similar in all animals as shown in Table 2. Five minutes after intraportal infusion of NE blood pressure increased significantly

Table 2. Initial blood pressure and blood pressure increment in control and cirrhotic rats following infusion of NE or saline

	No. of animals	Cirrhotic	Controls
Initial BP (mm Hg)	50	111.7 ± 2.12	123.6 ± 1.9
Increase in BP after NE infusion (mm Hg)	21	52.4 ± 2.6	48.8 ± 2.7
Increase in BP after saline infusion (mm Hg)	13	None	None

Values presented as mean ± SEM

Table 3. Portal pressure increment after intraportal infusion of NE or saline in cirrhotic and control animals

	No. of	Cirrhotic	Control
Increase in portal pressure after NE infusion (mm Hg)	5	4.8 ± 0.3	5.1 ± 0.6
Increase in portal pressure after saline infusion (mm Hg)	5	None	None

Values presented as mean ± SEM

Table 4. Bleeding time and blood loss at standardized liver trauma in cirrhotic and control animals following infusion of NE, saline or no infusion

Group	No. of animals	Bleeding time (s)			Blood loss (g)		
		Cirrhotic	Controls		Cirrhotic	Controls	
NE infusion	8	114 ± 8 s	89.6	s_1	2.8 ± 0.3 s	1.4 ± 0.1 s	
Saline infusion	8	259 ± 8 s	183 ± 12 s	s	4.9 ± 0.4 s	2.6 ± 0.1 s	
No infusion	8	277 ± 44 s	199 ± 12 s	s_1	5.6 ± 0.4 s	2.8 ± 0.2 s	

Significance of difference, P $\begin{matrix} s \leq 0.01 \\ s_1 \leq 0.05 \end{matrix}$

Values presented as mean ± SEM

Wilcoxon's rank sum test

by 52.4 mm Hg in cirrhotic animals and by 48.8 mm Hg in controls. Infusion of saline did not affect blood pressure.

Portal pressure increased significantly after infusion of NE in cirrhotic and control animals, the increment being 4.8 mm Hg and 5.1 mm Hg, respectively. There was no effect on portal pressure following infusion of saline (Table 3).

Bleeding time (s) and blood loss (g) during liver trauma were 277 ± 44 s and 5.6 ± 0.4 g in cirrhotic rats; significantly increased as compared with control values of 199 ± 12 s and 2.8 ± 0.2 g. Intraportal saline infusion did not affect these results. NE infusion reduced these parameters significantly. In cirrhotic rats bleeding time decreased to 114 ± 8 s and blood loss to 2.8 ± 0.3 g, whereas in controls bleeding time was 89 ± 6 s and blood loss 1.4 ± 0.1 g under the influence of NE (Table 4).

APT-time was prolonged in cirrhotic animals (70.3 ± 21.2 s) as compared with controls (27.1 ± 1.8 s). Infusion of NE did not alter APT-time in either cirrhotic or control rats (Table 5).

Table 5. APT-time (s) in control and cirrhotic animals following NE infusion or no infusion

Group	No. of animals	Cirrhotic	Control	
NE infusion	8	73.5 ± 21.7	28.8 ± 1.9	s
No infusion	8	70.3 ± 21.2 NS	27.1 ± 1.8 NS	s

Statistical significance, $P \begin{matrix} s \leq 0.01 \\ s_1 \leq 0.05 \end{matrix}$
Values presented as mean ± SEM
Wilcoxon's rank sum test

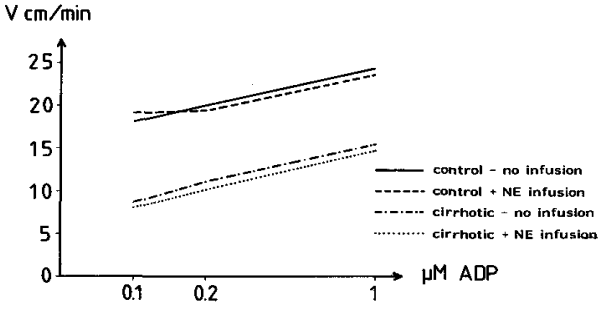

Fig. 3. Effect of NE infusion on platelet aggregation velocity at different ADP concentrations in control and cirrhotic rats

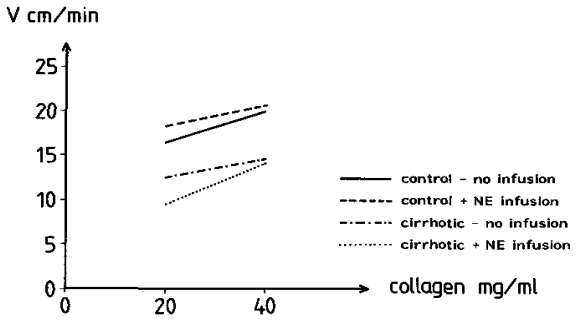

Fig. 4. Effect of NE infusion on platelet aggregation velocity at different collagen concentrations in controls and cirrhotic rats

No difference was found among cirrhotic and controls, concerning the measured hematologic parameters (Hb, Hct, PC, and WBC), except a definite but insignificant increase WBC in the cirrhotic groups. Serum protein, though decreased among cirrhotics, did not differ significantly from controls.

Figures 3 and 4 show platelet aggregation after ADP or collagen stimulation, in cirrhotic and control animals, following NE infusion. Significant decrease in platelet aggregation ($P < 0.01$) was observed in the cirrhotic groups. NE infusion did not affect platelet function.

Discussion

Norepinephrine was demonstrated to facilitate primary hemostasis during standardized hepatic lesion in cirrhotic rats. The observed effect might be due

either to the vasoconstricting properties of the agent or its direct action on thrombocytes.

Experimental induction of cirrhosis in rats by administration of CCl_4 and phenobarbitone was established by Proctor and Chatamra [5–7]. Phenobarbitone caused increase in liver size, smooth endoplasmic reticulum and cytochrome P450 activity [6]. CCl_4's acute toxicity related to binding with cytochrome P450 and was therefore enhanced by phenobarbitone [5]. In stock rats LD_{50} for CCl_4 was 5 ml/kg b.wt., while in the presence of phenobarbitone the LD_{50} dose was 0.5 ml/kg b.wt. Our results corresponded well with the findings of Proctor and Chatamra, yielding a 74% of histologically verified cirrhosis after 8–10 doses of CCl_4.

In the present study standardized liver trauma was used in the evaluation of the effect of i.p. infusion of NE on hepatic hemorrhage in cirrhotic rats. The above model was proven sensitive for the investigation of primary hemostasis in normal animals [8]. Standardization of the hepatic lesion was possible by the use of the tip of the left anterior lobe and, as the small difference of range (0.5%) indicated, easily applicable in all rats. NE a strong, a_1- and a_2-receptor agonist and weak b-receptor agonist has been considered more potent on veins than arteries [10].

According to Reilly et al. [11] in the normal rat a-receptors existed in all segments of hepatic microvasculature while b-receptors were isolated on portal venules and sinusoids. In sinusoids, the site responsive to adrenergic substances was demonstrated to be the lining cells of endothelium. Topical applications of NE on the liver resulted in enlargement of the cell nucleus which protruded into the lumen decreasing flow through the microvasculature.

As nervous regulation seemed to lack in sinusoids, the blood levels of adrenergic agents appeared to be the main factor regulating blood flow through hepatic microvasculature. An effect of physiological amounts of NE released by tyramine at the inlet and outlet sphincters was postulated to regulate blood flow in hepatic sinusoids [11–13].

Koo et al. [14] demonstrated dose-dependent response of sinusoids to adrenergic agents. Low doses (10^{-9}–10^{-7} M) of NE and tyramine constricted part of the sinusoids and dilatated others. At higher concentration (10^{-6}–10^{-4} M) constriction was general and attributed to the presence of a adrenergic mechanism. Dilatation was mediated by b-receptors in the hepatic microvascular bed.

To date, very little is known about the effect of adrenergic agents on cirrhotic liver. Cirrhosis resulted in the disorganizing of liver structure by formation of fibrous bands and nodules, but there was no evidence of interference with sinusoidal microarchitecture. However, as vessels were embedded in fibrous tissue their constrictive ability might be hindered leading to prolongation of hemorrhage upon injury.

Our findings clearly demonstrated that intraportal NE infusion reduce significantly oozing from the traumatized liver surface. The blood loss in infused cirrhotic animals was reduced to the normal control levels, while even more pronounced was the effect of NE on bleeding time, representing probably the effect of the drug on sinusoidal constriction. Infusion of NE resulted in an increase of portal pressure, indicating an effect upon liver vasculature.

Hb, Hct, WBC, PC, and serum protein, not differing significantly between cirrhotics and controls, were not influenced by the NE infusion. The intrinsic coagulation system in cirrhotic animals was defective as indicated by the prolonged ATP-time. It may be expected, therefore, that cirrhotic rats had an inadequate fibrin formation. Decreased platelet aggregation might explain the prolongation of bleeding time and blood loss in the presence of cirrhosis.

Infusion of NE did not affect intrinsic coagulation or platelet aggregation in the present study. It is suggested, therefore, that the effect of NE infusion on primary hemostasis is attributable to the constriction of the liver microvasculature.

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